ACUTE ORAL TOXICITY TO THE RAT

(ACUTE TOXIC CLASS METHOD)

Report

CONFIDENTIAL

[redacted]

[redacted]

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[redacted]

[redacted]

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COMPLIANCE WITH GOOD LABORATORY PRACTICE STANDARDS

ACUTE ORAL TOXICITY TO THE RAT

(ACUTE TOXIC CLASS METHOD)

The study described in this report was conducted in compliance with the following Good Laboratory Practice standards and, with the exception noted below, I consider the data generated to be valid.

The UK Good Laboratory Practice Regulations (Statutory Instrument 1999 No. 3106, as amended by Statutory Instrument 2004 No. 994.).

OECD Principles of Good Laboratory Practice (as revised in 1997), ENV/MC/CHEM(98)17.

EC Commission Directive 2004/10/EC of 11 February 2004 (Official Journal No. L 50/44).

Chemical analysis of formulated test articles for determination of stability, homogeneity and concentration was not undertaken for this study.

These principles of Good Laboratory Practice are accepted by the regulatory authorities of the United States of America and Japan on the basis of intergovernmental agreements.

.....
Date

QUALITY ASSURANCE STATEMENT

The following inspections and audits have been carried out in relation to this study:

Study Phase	Date(s) of Inspection	Date of Reporting to Study Director and Management
Protocol Audit	9 June 2004	9 June 2004
Report Audit	24 August 2004	24 August 2004

Process based inspections: At or about the time this study was in progress inspections of procedures employed on this type of study were carried out. These were conducted and reported to appropriate Company Management as indicated below:

Process Based Inspections	Date(s) of Inspection	Date of Reporting to Management
Animal receipt	3 March 2004	3 March 2004
Necropsy	14 April 2004	14 April 2004
Bodyweights	5 May 2004	5 May 2004
Dose administration	18 May 2004	18 May 2004
Clinical signs examination	18 May 2004	18 May 2004
Formulation procedures	20 May 2004	20 May 2004

In addition, an inspection of the facility where this study was conducted was carried out on an annual basis. These inspections were conducted and reported to Company Management.

Date

CONTRIBUTING SCIENTIST

SUMMARY

This study was performed to assess the acute oral toxicity of [redacted] the rat. The method followed was that described in:

EEC Methods for the determination of toxicity, Annex to Directive 96/54/EEC (Official Journal No. L248, 30.9.96), Part B, Method B.1 tris. Acute toxicity (oral) - acute toxic class method.

OECD Guideline for Testing of Chemicals No.423 'Acute Oral Toxicity - Acute Toxic Class Method' Adopted 17 December 2001.

EPA Health Effects Test Guidelines OPPTS. 870.1100 Acute Oral Toxicity. EPA 712-C-02-190, 2002.

Japanese Ministry of Agriculture, Forestry and Fisheries, Test Data for Registration of Agricultural Chemicals, Acute oral toxicity (2-1-1), 12 Nohsan No 8147, Agricultural Production Bureau, November 24, 2000.

A group of three fasted female rats received a single oral gavage dose of the test substance, formulated in [redacted] a dose level of 1000 mg/kg bodyweight. Results at this dosage indicated the acute lethal oral dose of the test material to be greater than 1000 mg/kg bodyweight. A further group of three fasted females was then similarly dosed at 1000 mg/kg to confirm the results at this dosage and complete the study.

All animals were killed as scheduled and examined macroscopically on Day 15, the end of the observation period.

There were no mortalities or clinical signs of reaction to treatment throughout the study.

Two females had low bodyweight gains on Day 15. All other animals were considered to have achieved satisfactory bodyweight gains throughout the study.

Abnormalities revealed at the macroscopic examination at study termination on Day 15 comprised congestion (characterised by blood vessels injected) of the caecum in one female.

Based on the limit of solubility of the test substance in the vehicle the maximum practical single dosage achieved on this study was 1000 mg/kg bodyweight.

The acute lethal oral dose (LD_{50}) to rats of [redacted] as therefore demonstrated to be greater than 1000 mg/kg bodyweight.

INTRODUCTION

This study was designed to assess the toxicity of [redacted] following a single oral dose in the rat. The rats were dosed by oral gavage as the test substance may be ingested accidentally.

The study was conducted in compliance with:

> EEC Methods for the determination of toxicity, Annex to Directive 96/54/EEC (Official Journal No. L248, 30.9.96), Part B, Method B.1 tris. Acute toxicity (oral) - acute toxic class method.
>
> OECD Guideline for Testing of Chemicals No.423 'Acute Oral Toxicity - Acute Toxic Class Method' Adopted 17 December 2001.
>
> EPA Health Effects Test Guidelines OPPTS. 870.1100 Acute Oral Toxicity. EPA 712-C-02-190, 2002.
>
> Japanese Ministry of Agriculture, Forestry and Fisheries, Test Data for Registration of Agricultural Chemicals, Acute oral toxicity (2-1-1), 12 Nohsan No 8147, Agricultural Production Bureau, November 24, 2000.

The acute toxic class method is a stepwise procedure, which uses three animals per step. Depending on the mortality and/or moribund status of the animals, on average two to four steps may be necessary to allow a judgement on the acute toxicity of the test substance. This procedure results in the use of substantially fewer animals than the more traditional LD_{50} study while allowing for acceptable data-based scientific conclusions. The acute toxic class method is based on biometric evaluations and has been subjected to international validation (Schlede *et al*, 1995).

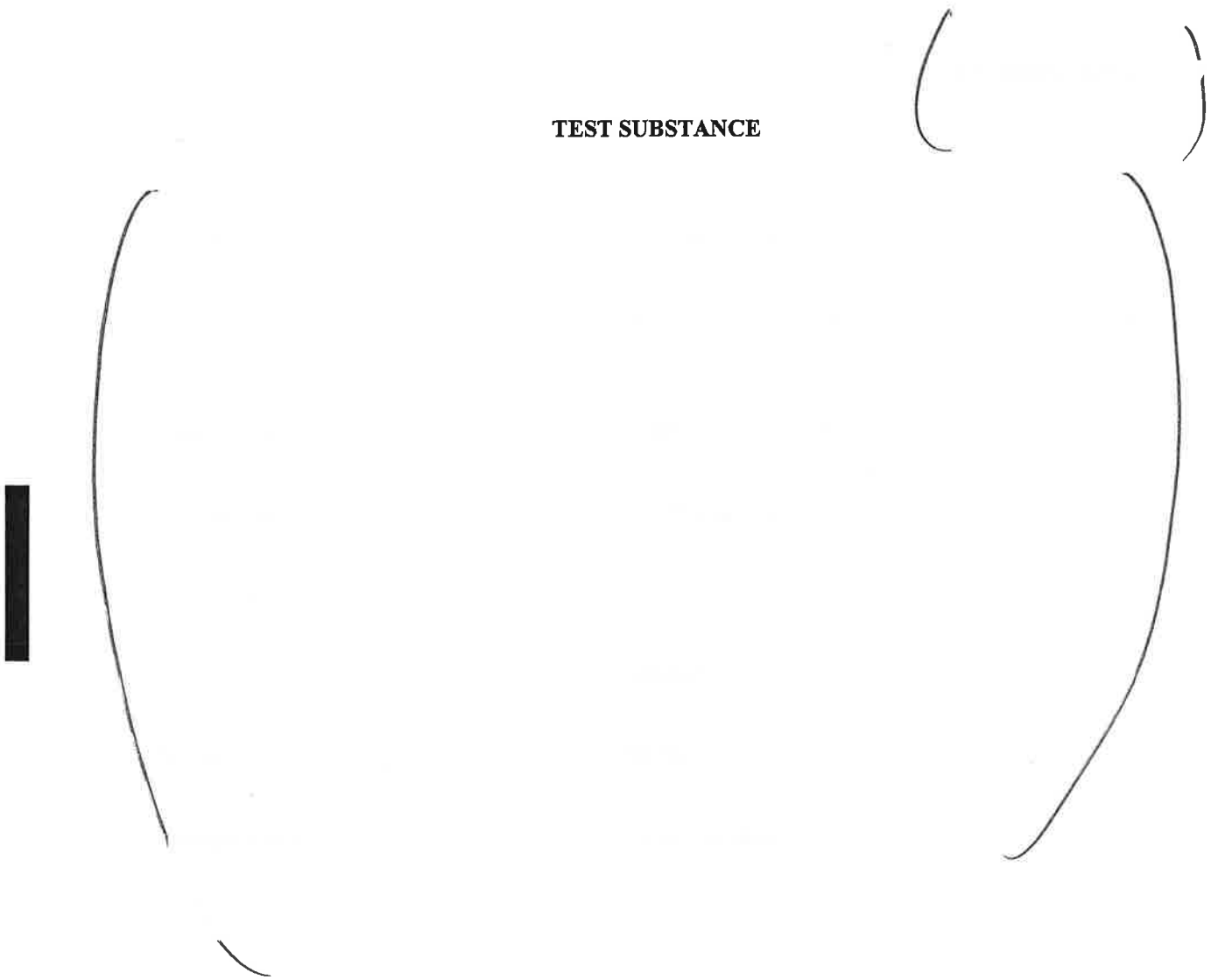
The rat was chosen as it has been shown to be a suitable model for this type of study and is the species recommended by the test guidelines.

The dose level for this study was chosen following consultation with the Sponsor. The dose level of 1000 mg/kg bodyweight was based on the limit of solubility of the test substance in the vehicl and potential toxicity of the vehicle.

The protocol was approved by [redacted] 7 April 2004, by the Sponsor on 16 April 2004 and by the Study Director on 3 June 2004.

The experimental phase of the study was undertaken betwee[redacted]

TEST SUBSTANCE



EXPERIMENTAL PROCEDURE

ANIMAL MANAGEMENT

Animals chosen for this study were selected from a stock supply of healthy female CD rats (which were nulliparous and non-pregnant) of Sprague-Dawley origin (Crl:CD BR) obtained from

They were in the weight range of 201 to 232 g and approximately eight to twelve weeks of age prior to dosing (Day 1). All the rats were acclimatised to the experimental environment for a minimum period of 13 days prior to the start of the study.

Rats were allocated without conscious bias to cages within the treatment groups. They were housed in groups of three rats of the same sex in metal cages with wire mesh floors

A standard laboratory rodent diet (Special Diet Services RM1(E) SQC expanded pellet) and drinking water were provided *ad libitum*. Access to food only was prevented overnight prior to and approximately 2¾ or 4 hours after dosing. Each batch of diet used for the study was analysed by the supplier for certain nutrients, possible contaminants and micro-organisms. Results of routine physical and chemical examination of drinking water, as conducted by the supplier are made available to Huntingdon Life Sciences Ltd. at regular intervals throughout the year.

During the acclimatisation period all animals were given small soft white untreated wooden blocks for environmental enrichment. These were removed from the cages on the day prior to dosing.

Animal room environmental controls were set to maintain temperature within the range 21 ± 2°C and relative humidity 40-70%. Any minor deviations from these ranges would not have had an adverse effect on the animals and would not affect the integrity or validity of the study. These environmental parameters were recorded daily and the permanent record archived with other departmental raw data. Lighting was controlled by means of a time switch to provide 12 hours of artificial light (0600 - 1800 GMT) in each 24-hour period.

Each animal was identified by tail marking. Each cage was identified by a coloured label displaying the dose level, study number and animal mark.

TEST SUBSTANCE PREPARATION

as formulated in DMSO at a concentration of 100 mg/ml and administered at a volume of 10 ml/kg bodyweight.

The test substance formulation was prepared on the day of dosing.

The absorption of the test substance was not determined.

Chemical analysis of formulated test articles for determination of stability, homogeneity and concentration was not undertaken for this study.

TREATMENT PROCEDURE

A group of three fasted female rats received a single oral gavage dose of the test substance, formulated in a dose level of 1000 mg/kg bodyweight. Results at this dosage indicated the acute lethal oral dose of the test material to be greater than 1000 mg/kg bodyweight. A further group of three fasted females was then similarly dosed at 1000 mg/kg to confirm the results at this dosage and complete the study.

The treatment regime and constitution of the groups are shown below:

Dates dosed	Dose (mg/kg)	Concentration (mg/ml)	Dose volume (ml/kg)	No. of rats Female
15.06.04	1000	100	10	3
17.06.04	1000	100	10	3

Control animals

No control animals were included in this study.

ADMINISTRATION OF THE TEST SUBSTANCE

The appropriate dose volume of the test substance was administered to each rat by oral gavage using a plastic syringe and rubber catheter (8 ch).

The day of dosing was designated Day 1.

OBSERVATIONS

Mortality

Cages of rats were checked at least twice daily for any mortalities.

Clinical signs

Animals were observed soon after dosing and at frequent intervals for the remainder of Day 1. On subsequent days, animals were observed once in the morning and again at the end of the experimental day (with the exception of Day 15 - morning only). The nature and severity of the clinical signs and time were recorded at each observation.

All animals were observed for 14 days after dosing.

Bodyweight

The bodyweight of each rat was recorded on Days 1 (prior to dosing), 8 and 15. Individual weekly bodyweight changes and group mean bodyweights were calculated.

TERMINAL STUDIES

Termination

All animals were killed on Day 15 by carbon dioxide asphyxiation.

Macroscopic pathology

All animals were subjected to a macroscopic examination which consisted of opening the cranial, thoracic and abdominal cavities. The macroscopic appearance of all examined organs was recorded.

ARCHIVES

All raw data arising from the performance of this study at is the property of the Sponsor and will be lodged together with a copy of the final report in the Archive.

Such records will be retained for a minimum of five years from the date on which the Study Director signs the final report. At the end of the five year retention period the Sponsor will be contacted and advice sought on the return, disposal or further retention of the records.

will retain the Quality Assurance records relevant to this study and a copy of the final report in its archive indefinitely.

DEVIATIONS FROM PROTOCOL

Animal numbers A4, A5 and A6 were re-fed approximately 2¾ hours following dosing and not after 4 hours as stated in the protocol.

This deviation from protocol has no impact on the integrity or validity of this study.

There were no other deviations from the protocol.

RESULTS

MORTALITY

There were no deaths during the study.

CLINICAL SIGNS

There were no clinical signs of reaction to treatment.

BODYWEIGHT (Tables 1 and 2)

Two females (Nos. A1 and A5) had low bodyweight gains on Day 15. All other animals were considered to have achieved satisfactory bodyweight gains throughout the study.

MACROSCOPIC EXAMINATION

Abnormalities revealed at the macroscopic examination at study termination on Day 15 comprised congestion (characterised by blood vessels injected) of the caecum in one female (No. A3).

CONCLUSION

Based on the limit of solubility of the test substance in the vehicle the maximum practical single dosage achieved on this study was 1000 mg/kg bodyweight.

The acute lethal oral dose (LD_{50}) to rats of [redacted] was therefore demonstrated to be greater than 1000 mg/kg bodyweight.

TABLE 1

Individual and group mean bodyweights (g)

Dose (mg/kg)	Sex	Animal Number	Bodyweight (g) at Day		
			1*	8	15
1000	Female	A1	220	248	252
		A2	203	230	241
		A3	222	262	271
		Mean	215	247	255
		A4	228	253	271
		A5	232	275	279
		A6	201	218	235
		Mean	220	249	262

* Prior to dosing

TABLE 2

Individual bodyweight changes (g)

Dose (mg/kg)	Sex	Animal Number	Bodyweight gains (g) at Day	
			8	15
1000	Female	A1	28	4
		A2	27	11
		A3	40	9
		A4	25	18
		A5	43	4
		A6	17	17

APPENDIX 1

References

EEC Methods for the determination of toxicity, Annex to Directive 96/54/EEC (Official Journal No. L248, 30.9.96), Part B, Method B.1 tris. Acute toxicity (oral) - Acute Toxic Class Method.

EPA Health Effects Test Guidelines OPPTS. 870.1100 Acute Oral Toxicity. EPA 712-C-02-190, 2002.

OECD Guideline for Testing of Chemicals No. 423 'Acute Oral Toxicity - Acute Toxic Class Method'. Adopted 17 December 2001.

SCHLEDE, E., MISCHKE, U., DIENER, W., and KAYSER, D. (1995). The International Validation Study of the Acute-Toxic-Class Method (Oral). *Arch Toxicol.* 69, 659-670.

Japanese Ministry of Agriculture, Forestry and Fisheries, Test Data for Registration of Agricultural Chemicals, Acute oral toxicity (2-1-1), 12 Nohsan No 8147, Agricultural Production Bureau, November 24, 2000.

OECD (1998) Harmonised Integrated Hazard Classification System for Human Health and Environmental Effects of Chemical Substances as endorsed by the 28th Joint Meeting of the Chemicals Committee and the Working Party on Chemicals in November 1998, Part 2, p.11.

OECD (2000a) Guidance document on Acute Oral Toxicity. Environmental Health and Safety Monograph Series on Testing and Assessment No. 24.

OECD (2000b) Guidance document on the Recognition, Assessment and use of clinical signs as Humane Endpoints for Experimental animals used in Safety Evaluation Environmental Health and Safety Monograph series on Testing and Assessment No. 19.

HUNTINGDON RESEARCH CENTRE GLP COMPLIANCE STATEMENT 2003

THE DEPARTMENT OF HEALTH OF THE GOVERNMENT OF THE UNITED KINGDOM

GOOD LABORATORY PRACTICE

STATEMENT OF COMPLIANCE
IN ACCORDANCE WITH DIRECTIVE 88/320 EEC

LABORATORY

TEST TYPE

Analytical Chemistry
Clinical Chemistry
Ecosystems
Environmental Fate
Environmental Toxicity
Toxicology
Phys/Chem Tests

DATE OF INSPECTION

07th April 2003

A general inspection for compliance with the Principles of Good Laboratory Practice was carried out at the above laboratory as part of UK GLP Compliance Programme.

At the time of the inspection no deviations were found of sufficient magnitude to affect the validity of non-clinical studies performed at these facilities.

Roger G. Alexander 1/8/03

Dr. Roger G. Alexander
Head, UK GLP Monitoring Authority

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Total number of pages: 2

Number of pages for internal distribution: 2

Study Director

The signature of the Study Director authorises the implementation of this amendment to protocol. In this amendment, deleted statements are struck through and new statements are underlined. Any changes to the study design after the date of this authorising signature will be documented in a further formal amendment.

AMENDMENT APPROVAL

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Reasons for amendment

1: Protocol: Study details page amended (Proposed study dates and Modifications section page 1). Study start delayed due to difficulties with formulating the test substance. Due to the limit of solubility of the test substance in the proposed vehicle (111 mg/ml in e maximum dose level administered on this study will be 1000 mg/kg bodyweight.

Amendment

Study details page (1)

Proposed study dates

Start:	Week beginning ~~7~~ 14 June 2004
Completion:	Week beginning ~~5~~ 12 July 2004
Draft report:	~~3~~ 10 September 2004

Modifications:

The EC Commission Directive has been updated to:

EC Commission Directive 2004/10/EC of 11 February 2004 (Official Journal No L 50/44).

The maximum dose level on this study will be 1000 mg/kg bodyweight.